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(54) Title: MICROFLUIDIC SURFACES

(57) Abstract: A microfluidic device comprising a set of one or more, preferably more than 5, covered microchannel structures manufactured in the surface of a planar substrate. The device is characterized in that a part surface of at least one of the microchannel structures has a coat exposing a non-ionic hydrophilic polymer. The non-ionic hydrophilic polymer is preferably attached covalently directly to the part surface or to a polymer skeleton that is attached to the surface.

MICROFLUIDIC SURFACES

Technical field

The invention concerns a microfluidic device comprising a set of one or more, preferably more than 5, covered microchannel structures fabricated in the surface of a planar substrate.

By the term "covered" is meant that a lid covers the microchannel structures thereby minimising or preventing undesired evaporation of liquids. The cover/lid may have microstructures matching each microchannel structure in the substrate surface.

The term "fabricated" means that two-dimensional and/or three-dimensional microstructures are present in the surface. The difference between a two-dimensional and a three-dimensional microstructure is that in the former variant there are no physical barriers delineating the structure while in the latter variant there are. See for instance WO 9958245 (Larsson et al).

The part of the cover/lid, which is facing the interior of a microchannel is included in the surface of a microchannel structure.

The planar substrate typically is made of inorganic and/or organic material, preferably of plastics. For examples of various inorganic and organic materials see under the heading "Material in the microfluidic device".

A microfluidic device encompasses that there is a liquid flow that causes mass transport of solutes and/or particles dispersed in the liquid from one functional part of the structure to another. Sole capillaries, possibly with an area for application and an area for detection, as used in capillary electrophoresis in which solutes are caused to migrate by an applied electric field for separation purposes are not microfluidic devices as contemplated in the context of the invention. An electrophoresis capillary may, however, be part of a microfluidic device if the capillary is part of a microchannel structure in which there are one or more additional functional parts from and/or to which mass transport of a solute by a liquid flow is taking place as defined above.

The liquid is typically polar, for instance aqueous such as water.

Technical background.

Microfluidic devices require that liquid flow easily pass through the channels and that non-specific adsorption of reagents and analytes should be as low as possible, i.e. insignificant for the reactions to be carried out.

Reagents and/or analytes includes proteins, nucleic acids, carbohydrates, cells, cell particles, bacteria, viruses etc. Proteins include any compound exhibiting poly- or oligopeptide structure.

The hydrophilicity of surfaces within microchannel structures shall support reproducible and predetermined penetration of an aqueous liquid into the various parts of a structure. It is desirable that once the liquid has passed a possible break at the entrance of a part of the structure then the liquid spontaneously shall enter the part by capillary action (passive movement). This in turn means that the hydrophilicity of the surfaces within microchannel structures becomes of increasing importance when going from a macroformat to a microformat.

From our experience, water contact angles around 20 degrees or lower may often be needed to accomplish reliable passive fluid movement into microchannel structures. However, it is not simple to manufacture surfaces which permanently have such low water contact angles. There is often a tendency for a change in water contact angles during storage, which renders it difficult to market microfluidic devices having standardised flow properties.

The situation is complicated by the fact that methods for preparing surfaces with very low water contact angles do not necessarily reduce the ability to non-specifically adsorb reagents and sample constituents. The surface/volume ratio increases when going from a macroformat down to smaller formats. This means that the capacity for non-specific adsorption of a surface increases inversely with the volume surrounded by the surface. Non-specific adsorption therefore becomes more critical in microformat devices than in larger devices.

An unacceptable non-specific adsorption of biomolecules is often associated with the presence of hydrophobic surface structures. This particular problem therefore is often more severe in relation to surfaces made of plastics and other hydrophobic materials compared to surfaces of native silicon surfaces and other similar inorganic materials.

There are a number of methods available for treating surfaces to make them hydrophilic in order to reduce non-specific adsorption of various kinds of biomolecules and other reagents. However, these methods generally do not concern balancing a low non-specific adsorption with a reliable and reproducible liquid flow when miniaturizing macroformats down into microformats. Compare for instance Elbert et al., (Annu. Rev. Mater. Sci. 26 (1996) 365-394).

Surfaces that have been rendered repelling for biopolymers in general by coating with adducts between polyethylenimines and hydrophilic polymers have been described during the last decade (Brink et al (US 5,240,994), Bergström et al., US 5,250,613; Holmberg et al., J. Adhesion Sci. Technol. 7(6) (1993) 503-517; Bergström et al., Polymer Biomaterials, Eds Cooper, Bamfors, Tsuruta, VSP (1995) 195-204; Holmberg et al., Mittal Festschrift, Eds Van Ooij, Anderson, VSP 1998, p 443-460; and Holmberg et al., Biopolymers at Interfaces, Dekker 1998 (Surfactant Science Series 75), 597-626). Sequential attachment of a polyethylenimine and a hydrophilic polymer has also been described (Kiss et al., Prog. Colloid Polym. Sci. 74 (1987) 113-119).

Non-specific adsorption and/or electroendosmosis have been controlled in capillary electrophoresis by coating the inner surface of the capillary used with a hydrophilic layer, typically in form of a hydrophilic polymer (e.g. van Alstine et al US 4,690,749; Ekström & Arvidsson WO 9800709; Hjertén, US 4,680,201 (poly methacrylamide); Karger et al., US 5,840,388 (polyvinyl alcohol (PVA)); and Soane et al., US 5,858,188 and US 6,054,034 (acrylic microchannels). Capillary electrophoresis is a common name for separation techniques carried out in a narrow capillary utilizing an applied electric filed for mass transport and separation of the analytes.

Larsson et al (WO 9958245, Amersham Pharmacia Biotech) presents among others a microfluidic device in which microchannels between two planar substrates are defined by the interface between hydrophilic and hydrophobic areas in at

least one of the substrates. For aqueous liquids the hydrophilic areas define the fluid pathways. Various ways of obtaining a pattern of hydrophobic and hydrophilic surfaces for different purposes are discussed, for instance, plasma treatment, coating a hydrophobic surfaces with a hydrophilic polymer etc. The hydrophilic coat polymers suggested may or may not have aryl groups suggesting that Larsson et al are not focusing on lowering the water contact angle as much as possible or avoiding non-specific adsorption.

Larsson, Ocklind and Derand (PCT/EP00/05193 claiming priority from SE 9901100-9, filed 1999-03-24) describe the production of highly hydrophilic surfaces made of plastics. The surfaces retain their hydrophilicity even after being in contact with aqueous liquids. An additional issue in PCT/EP00/05193 is to balance a permanent hydrophilicity with good cell attachment properties. The surfaces are primarily suggested to be used in microfabricated devices.

Polyethylene glycol has been linked directly to the surface of a microchannel fabricated in silicone for testing the ability of polyethylyne glycol to prevent protein adsorption. See Bell, Brody and Yager (SPIE-Int. Soc. Opt. Eng. (1998) 3258 (Micro- and Nanofabricated Structures and Devices for Biomedical Environmental Applications) 134-140).

The objectives of the invention.

A first objective is to accomplish a sufficiently reliable and reproducible mass transport of reagents and sample constituents (e.g. analytes) in microfluidic devices.

A second objective is to enable a reliable and reproducible aqueous liquid flow in the microfluidic devices.

A third objective is to optimise non-specific adsorption and hydrophilicity in relation to each other for surfaces of fluid pathways in microfluidic devices.

The invention

We have discovered that by attaching a hydrophilic non-ionic polymer to the surface of a microchannel structure in a microfluidic device one can easily minimize the above-mentioned problems also for the most critical surface materials. This discovery facilitates creation of surfaces that permit reliable and reproducible transport of reagents and sample constituents in microfluidic devices.

The main aspect of the invention is a microfluidic device as defined under the heading "Technical Field". The characterizing feature is that at least a part surface of each microchannel structure exposes a firmly attached non-ionic hydrophilic polymer to the interior of the structure.

The non-ionic hydrophilic polymer may be attached directly to the surface of the microchannel structure or via a polymer skeleton that in turn is attached to the surface via multipoint attachment.

The non-ionic hydrophilic polymer

The non-ionic hydrophilic polymer contains a plurality of hydrophilic neutral groups. Neutral groups excludes non-charged groups that can be charged by a pH-change. Typical neutral hydrophilic groups contains an heteroatom (oxygen, sulphur or nitrogen) and may be selected among hydroxy, ether such as ethylene oxy (e.g. in polyethylene oxide), amides that may be N-substituted etc. The polymer as such is also inert towards the reagents and chemicals that are to be used in the microfluidic device.

Illustrative non-ionic hydrophilic polymers are preferably water-soluble when not bound to a surface. Their molecular weight is within the range from about 400 to about 1,000,000 daltons, preferably from about 1,000 to about 2000,000, such as below 100,000 daltons.

Non-ionic hydrophilic polymers are illustrated with polyethylene glycol, or more or less randomly distributed or block-distributed homo- and copolymers of lower alkylene oxides (C_{1-10}, such as C_{2-10}) or lower alkylene (C_{1-10}, such as C_{2-10}) bisepoxides in which the epoxide groups are linked together via a carbon chain comprising 2-10 sp^3-carbons. The carbon chain may be interrupted at one or more positions by an ether oxygen, i.e. an ether oxygen is inserted between two carbon atoms. A hydrogen atom at one or more of the methylene groups may be replaced with hydroxy groups or lower alkoxy groups (C_{1-4}). For stability reasons at most one oxygen atom should be bound to one and the same carbon atom.

Other suitable non-ionic hydrophilic polymers are polyhydroxy polymers that may be completely or partly natural or completely synthetic.

Completely or partly natural polyhydroxy polymers are represented by polysaccharides, such as dextran and its water-soluble derivatives, water-soluble derivatives of starch, and water-soluble derivatives of cellulose, such as certain cellulose ethers. Potentially interesting cellulose ethers are methyl cellulose, methyl hydroxy propyl cellulose, and ethyl hydroxy ethyl cellulose.

Synthetic polyhydroxy polymers of interest are also polyvinyl alcohol possibly in partly acetylated form, poly(hydroxy lower alkyl vinyl ether) polymers, polymers obtained by

polymerisation of epichlorohydrin, glycidol and similar bifunctionally reactive monomers giving polyhydroxy polymers.

Polyvinylpyrrolidone (PVP), polyacrylamides, polymethacrylamides etc are examples of polymers in which there are a plurality of amide groups.

Further suitable hydrophilic polymers are reaction products (adducts) between ethylene oxide, optionally in combination with higher alkylene oxides or bisepoxides, or tetrahydrofuran, and a dihydroxy or polyhydroxy compound as illustrated with glycerol, pentaerythritol and any of the polyhydroxy polymers referred to in the preceding paragraphs.

The non-ionic hydrophilic polymer may have the same structure as described for the extenders defined in Berg et al (WO 9833572) which is hereby incorporated by reference. In contrast to Berg et al there is no imperative need for the presence of an affinity ligand on the hydrophilic polymer used in the present invention.

One or more positions in the non-ionic hydrophilic polymer may be utilized for attachment. In order to make the hydrophilic polymer flexible the number of attachment points should be as low as possible, for instance one, two or three positions per polymer molecule. For straight chain polymers, such as lower alkylene oxide polymers similar to polyethylene oxide, the number of attachment points is typically one or two, with preference for one.

Depending on the position of a coated part surface within a microchannel structure, the hydrophilic polymer may carry an immobilized reactant (often called ligand when affinity reactions are concerned). Depending on the particular use of a microchannel structure such reactants can be so called

affinity reactants that are used to catch an analyte or an added reactant or a contaminant present in the sample. Immobilized ligands also include immobilized enzymes. According to the invention this kind of reactants are preferably present in reaction chambers/cavities (see below).

The skeleton

The skeleton may be an organic or inorganic cationic, anionic or neutral polymer of inorganic or organic material.

With respect to inorganic skeletons, the preferred variants are polymers such as silicon oxide. See the experimental part.

With respect to organic skeletons, the preferred variants are cationic polymers, such as a polyamine, i.e. a polymer containing two or more primary, secondary or tertiary amine groups or quaternary ammonium groups. The preferred polyamines are polyalkylenimines, i.e. polymers in which amine groups are interlinked by alkylene chains. The alkylene chains are for instance selected among C_{1-6} alkylene chains. The alkylene chains may carry neutral hydrophilic groups, for instance hydroxy (HO) or poly (including oligo) lower alkylene oxy groups [$-O-((C_2H_4)_nO)_mH$ where n is 1-5 and m is from 1 and upwards for instance ≤ 100 or ≤ 50)], amide groups, acyl, acyloxy, lower alkyl (for instance C_{1-5}) and other neutral groups and/or groups that are unreactive under the conditions to be applied in the microfluidic device.

The preferred molecular weight of the skeleton including polyamine skeletons is within the range of 10,000-3,000,000 daltons, preferably about 50,000-2,000,000 daltons. The structure of the skeleton can be linear, branched, hyperbranched or dendritic. The preferred polyamine skeleton is polyethylenimine, a compound that is achievable e.g. by polymerizing ethylene imine, usually giving hyperbranched chains.

Attachment of the non-ionic hydrophilic polymer

The introduction of the non-ionic hydrophilic polymer groups on the channel surfaces may be done according to principles well-known in the field, forinstance by directly attaching the hydrophilic polymer to the desired part surface or via the kind of skeleton discussed above. The adduct between the skeleton and the non-ionic hydrophilic polymer may be (i) formed separately before it is attached to the surface or (ii) on the surface by first attaching the skeleton and then the hydrophilic polymer. Alternative (ii) can be carried out by (a) grafting a preprepared non-ionic hydrophilic polymer to the skeleton or (b) graft polymerisation of suitable monomers.

Both the non-ionic hydrophilic polymer and the skeleton may be stabilized to the underlying surfaces via covalent bonds, electrostatic interaction etc and/or by cross-linking *in situ* or afterwards. A polyamine skeleton, for instance, may be attached covalently by reacting its amine functions with aminereactive groups that are originally present or have been introduced on the uncoated substrate surface.
It is important that the nude part surface to be coated according to the invention has groups, which enable stable interaction between the non-ionic hydrophilic polymer and the surface and between the skeleton and the surface. Cationic skeletons, for instance polyamines, require that negatively charged or chargeable groups or groups otherwise capable of binding to amine groups, typically hydrophilic, are exposed on the surface. Polar and/or charged or chargeable groups may easily be introduced on plastics surfaces, for instance by treatment with O_2- and acrylic acid-containing plasmas, by oxidation with permanaganate or bichromate in concentrated sulphuric acid, by coating with polymers containing these type

of groups etc. In other words by techniques well-known in the scientific and patent literature. The plastics surface as such may also contain this kind of groups without any pretreatment, i.e. by being obtained from polymerisation of monomers either carrying the above-mentioned type of groups or groups that subsequent to polymerisation easily can be transformed to such groups.

If the surface to be coated is made of a metal, for instance of gold or platina, and the non-ionic hydrophilic polymer or skeleton has thiol groups, attachment can be accomplished via bonds that are partly covalent.

If the non-ionic hydrophilic polymer or the skeleton have hydrocarbon groups, for instance pure alkyl groups or phenyl groups, one can envisage that attachment to the substrate surface can take place via hydrophobic interactions.

Water contact angles

The optimal water contact angle depends on the analyses and reactions to be carried out in the microchannel structure, dimensions of the microchannels and chambers of the structures, composition and surface tension of liquids used, etc. As a rule of thumb, the inventive coat should be selected to provide a water contact angle that is ≤ 30°, such as ≤ 25° or ≤ 20°. These figures refer to values obtained at the temperature of use, primarily room temperature.

So far the most superior surfaces have been those based on adducts between polyethylene imine and polyethylene glycol with monosite (mono group terminal) attachment of the non-ionic hydrophilic polymer to the polyethylene imine skeleton. The best mode to date of this preferred variant is given in the experimental part (example 1).

Thickness of the coat

The thickness of the hydrated coat provided by the non-ionic hydrophilic polymers should be ≤ 50 %, for instance ≤ 20 % of the smallest distance between two opposing sides of a part of the microchannel structure comprising the surface coated according to the invention. This typically means that an optimal thickness will be within the interval 0.1-1000 nm, for instance 1-100 nm, with the provision that the coat shall permit a desired flow to pass through.

Structures in the microfluidic device.

The microfluidic device may be disc-formed of various geometries, with the round form being the preferred variant (CD-form).

On devices having round forms, the microchannel structures may be arranged radially with an intended flow direction from an inner application area radially towards the periphery of the disc. In this variant the most practical ways of driving the flow is by capillary action, centripetal force (spinning the disc) and/or hydrodynamically.

Each microchannel structure comprises one or more channels and/or one or more cavities in the microformat. Different parts of a structure may have different discrete functions. Thus there may be one or more parts that function as (a) application chamber/cavity/area (b) conduit for liquid transport, (c) reaction chamber/cavity, (d) volume defining unit, (e) mixing chamber/cavity, (f) chamber for separating components in the sample, for instance by capillary electrophoresis, chromatography and the like (g) detection chamber/cavity, (h) waste conduit/chamber/cavity etc. According to the invention at least one of these parts may have the inventive coat on its surface, i.e. corresponds to the part surface discussed above.

When the structure is used, necessary reagents and/or sample including the analyte are applied to an application area and transported downstream in the structure by an applied liquid flow. Some of the reagents may have been predispensed to a chamber/cavity. The liquid flow may be driven by capillary forces, and/or centripetal force, pressure differences applied externally over a microchannel structure and also other non-electrokinetic forces that are externally applied and cause transport of the liquid and the analytes and reagents in the same direction. The liquid flow may also be driven by pressure generated by electroendoosmosis created within the structure. The liquid flow will thus transport reagents and analytes and other constituents from an application area/cavity/chamber into a sequence comprising a particular order of preselected parts (b)-(h). The liquid flow may be paused when a reagent and/or analyte have reached a preselected part in which they are subjected to a certain procedure, for instance capillary electrophoresis in a separation part, a reaction in a reaction part, detection in a detection part etc.

Analytical and preparative methods as discussed below utilizing the microfluidic device of the invention with transport of liquid, reagents and analytes as described in the preceding paragraph constitute a separate aspect of the invention.

Microformat means that at least one liquid conduit in the structure has a depth and/or width that is in the microformat range, i.e. $< 10^3$ µm, preferably $< 10^2$ µm. Each microchannel structure extends in a common plane of the planar substrate material. In addition there may be extensions in other directions, primarily perpendicular to the common plane. Such other extensions may function as sample or liquid application areas or connections to other microchannel structures that are not located in the common plane, for instance.

The distance between two opposite walls in a channel is ≤ 1000 μm, such as ≤ 100 μm, or even ≤ 10 μm, such as ≤ 1 μm. The structures may also contain one or more chambers or cavities connected to the channels and having volumes being ≤ 500 μl, such as ≤ 100 μl and even ≤ 10 μl such as ≤ 1 μl. The depths of the chambers/cavities may typically be in the interval ≤ 1000 μm such as ≤ 100 μm such as ≤ 10 μm or even ≤ 1 μm. The lower limit is always significantly greater than the largest of the reagents used. The lower limits of chambers and channels are typically in the range 0.1-0.01 μm for devices that are to be delivered in dry form.

It is believed that the preferred variants of the inventive microfluidic devices will be delivered to the customer in a dried state. The surfaces of the microchannel structures of the device therefore should have a hydrophilicity sufficient to permit the aqueous liquid to be used to penetrate the different parts of the channels of the structure by capillary forces (self-suction).

There may be conduits enabling liquid communication between individual microchannel structures within a set.

Material in the microfluidic device.
The surface to be coated according to the invention typically is made of inorganic and/or organic material, preferably of plastics. Diamond material and other forms of elemental carbon are included in the term organic material. Among suitable

inorganic surface materials can be mentioned metal surfaces, e.g. made of gold, platina etc.

Plastics to be coated according to the invention may have been obtained by polymerisation of monomers comprising unsaturation such as carbon-carbon double bonds and/or carbon-carbon-triple bonds.

The monomers may, for instance, be selected from mono-, di and poly/oligo-unsaturated compounds, e.g. vinyl compounds and other compounds containing unsaturation. Illustrative monomers are:

(i) alkenes/alkadienes (such as ethylene, butadiene, propylene and including substituted forms such as vinyl ethers), cycloalkenes, polyfluorovinyl hydrocarbons (for instance tetrafluoroethylene), alkene-containing acids, esters, amides, nitriles etc for instance various methacryl/acryl compounds; and

(ii) vinyl aryl compounds (such as mono-, di- and trivinyl benzenes) that optionally may be substituted with for instance lower alkyl groups (C1-6) etc.

Another type of plastics are based on condensation polymers in which the monomers are selected from compounds exhibiting two or more groups selected among amino, hydroxy, carboxy etc groups. Particularly emphasised monomers are polyamino monomers, polycarboxy monomers (including corresponding reactive halides, esters and anhydrides), poly hydroxy monomers, amino-carboxy monomers, amino-hydroxy monomers and hydroxy-carboxy monomers, in which poly stands for two, three or more functional groups. Polyfunctional compounds include compounds having a functional group that is reactive twice, for instance carbonic acid or formaldehyde. The plastics contemplated are typically polycarbonates, polyamides, polyamines, polyethers etc. Polyethers include the corresponding silicon analogues, such as silicone rubber.

The polymers of the plastics may be in cross-linked form.

The plastics may be a mixture of two or more different polymer(s)/copolymer(s).

Particularly interesting plastics are those that have a non-significant fluorescence for excitation wavelengths in the interval 200-800 nm and emission wavelengths in the interval 400-900 nm. By non-significant fluorescence is meant that the fluorescence intensity in the above-given emission wavelength interval should be below 50 % of the fluorescence intensity for a reference plastics (= a polycarbonate of bisphenol A without fluorescent additives). In fact it does not harm in case the fluorescence intensity of the plastics is even lower, such as < 30 % or < 15 %, such as < 5 % or < 1 %, of the fluorescence intensity of the reference plastics. Typical plastics having an acceptable fluorescence are based on polymers of aliphatic monomers containing polymerizable carbon-carbon double bonds, such as polymers of cykloalkenes (e.g. norbornene och substituted norbornenes), ethylene, propylenes etc, as well as other non-aromatic polymers of high purity, e.g. certain grades of polymethylmethacrylate.

In preferred variants of the invention the same limits for fluorescence also apply to the microfluidic structure after having been coated in accordance with the invention.

Applications in which the inventive microfluidic device can be used.

The primary use of the microfluidic devices of the invention is in analytical and preparative chemical and biochemical systems.

Typical analytical systems in which the microfluidic systems described herein may comprise as the main steps one or more of (a) sample preparation, (b) assay reactions and (c) detection. Sample preparation means the preparation of a sample in order to make it suitable for the assay reactions and/or for the detection of a certain activity or molecular entity. This may for example mean that substances interfering with the assay reactions and/or detection is removed or otherwise neutralized, that substances are amplified and/or derivatized etc. Typical examples are (1) amplifying one or more nucleic acid sequences in a sample, for instance by polymerase chain reaction (PCR), (2) removing of species cross-reacting with an analyte in assays involving affinity reactions etc. Typical assay reactions are (i) reactions involving cells, (ii) affinity reactions, for instance biospecific affinity including immune reactions, enzymatic reactions, hybridization/annealing etc, (iii) precipitation reactions, (iv) pure chemical reactions involving formation or breaking up of covalent bonds, etc. The detection reaction may involve fluorometry, chemiluminometry, mass spectrometry, nephelometry, turbidometry etc. The detection reaction aims at detection of the result of the assay reaction(s) and at relating a found result with the qualitative or quantitative presence of an activity in the original sample. The activity can be a biological, a chemical, a biochemical etc activity. It may be as the presence of a compound as such or simply as an activity of a known or unknown compound. If the system is used for diagnostic purposes the result in the detection step is further correlated to the medicinal status of the individual from which the sample derives. The applicable analytical systems may thus comprise affinity assays, such as immune assays, hybridisation assays, cell biology assays, mutation detection, genome characterisation, enzyme assays, screening assays for finding new affinity pairs etc. Methods for the analysis of sample content of proteins, nucleic acids, carbohydrates, lipids and other molecules with particular emphasis of other bio-organic molecules are also included.

The microfluidic device of the present invention may also find use for the set up of libraries of compounds including synthetic peptide and oligonucleotide libraries, for instance by solid phase synthesis. The synthesis of so called combinatorial libraries of compounds is also included.

The invention will now be described with reference to non-limitative experiments that function as proof of principle.

EXPERIMENTAL PART

A. COAT OF PEG-PEI ADDUCT

a. Synthesis of PEG-PEI adduct

0.43 g of polyethylenimine (Polymin SN from BASF, Germany) was dissolved in 45 ml of 50 mM sodium borate buffer (pH 9.5) at 45°C. 5 g of the glycidyl ether of monomethoxy polyethylene glycol (Mw 5 000) was added during stirring and the mixture was stirred for 3 h at 45°C.

b. Surface treatment

A polycarbonate CD disc (polycarbonate of Bisphenol A, Macrolon DP-1265, Bayer AG, Germany) with a recessed microchannel pattern was placed in a plasma reactor (Plasma Science PS0500, BOC Coating Technology, USA) and treated with an oxygen plasma at 5 sccm gas flow and 500 W RF power for 10 min. After venting the reactor, the disc was immersed in a 0.1% solution of the PEG-PEI adduct in borate buffer pH 9.5 for 1 h. The disc was then rinsed with distilled water, blown dry with nitrogen and the water contact angle (sessile drop) was measured on a Ramé-Hart manual goniometer bench. The average of six equilibrium measurements (three droplets) was

24 degrees. An XPS spectrum of the treated surface gave the following molar elemental composition: 73.2% C, 3.7 % N, 23.1% O, showing that the surface was essentially covered by the adsorbed PEG-PEI adduct.

c. Capillary wetting

Another polycarbonate CD disc of the same material as above with a recessed microchannel pattern was treated as in example 2. It was then covered with a thin silicone rubber lid, with a hole placed over a microchannel. When a droplet of water was placed in the hole with a micropipette, the water was drawn in by capillary forces and penetrated the entire accessible channel system.

d. Comparative examples of surface treatments

a) A polycarbonate disc of the same material as above with a recessed microchannel pattern was dipped into a 0.5% water solution of phenyl dextran (degree of substitution: 0.2 per monosacharide unit of dextran, Mw 40 000) for 1 h. After water rinsing, the disc was blown dry with nitrogen. The water contact angle was 30 degrees. When a silicone rubber lid was placed over the disc with a hole over a channel, the droplet was not spontaneously drawn in. When a vacuum was applied to the channel through another hole in the lid, the droplet could however be introduced by suction.

b) A polycarbonate disc of the same material as above with a recessed microchannel pattern was immersed over night in a 1 % water solution of a polyethylene glycol "polypropylene glycol" polyethylene glycol triblock copolymer (Pluronic F108 from BASF). After water rinsing the disc was blown dry with nitrogen. The water contact angle was 60 degrees. When a silicone rubber lid was placed over the disc with a hole over a channel, the droplet was not spontaneously drawn in. When a vacuum was applied to the channel through another hole in the lid, the droplet could however be introduced by suction.

B. POLY(ACRYLAMIDE) COATING.

a) Activation of the surface.

A PET foil (polyethylene terephthalate, Melinex®, ICI), evaporation coated with a thin film of silicon oxide, was used as a lid. The silicon oxide side of the PET foil was washed with ethanol and thereafter UV/Ozone (UVO cleaner, Model no 144A X-220, Jelight Company, USA) treated for 5 minutes. 15 mm Bind silane (3-methacryloloxypropyl trimethoxysilane, Amersham Pharmacia Biotech), 1.25 ml 10% acetic acid and 5 ml ethanol was mixed and thereafter applied onto the foil using a brush. After evaporation of the solvent, the foil was washed with ethanol and blown dry with nitrogen. The water contact angle (sessile drop) was measured on a Ramé-Hart manual goniometer. The average of repeated measurements was 62 degrees.

b. Grafting polyacrylamide to the activated surface

8.5 ml of 3 M acrylamide in water and 1.5 ml of 100 mM Irgacure 184 (dissolved in ethylene glycol, Ciba-Geigy) was mixed. The resulting solution was spread out on a quartz plate, and the activated PET foil was placed on top. The monomer solution was UV illuminated for 20 minutes through the quartz plate. The PET foil was then washed thoroughly in water and the average contact angle of repeated measurements was 17 degrees.

c. Capillary wetting

A piece of room temperature vulcanizing silicone rubber (Memosil, Wacker Chemie) having a microchannel structure and two holes was placed onto the polyacrylamide grafted PET foil (lid) (according to b above). When a droplet of water was placed in the hole with a micropipette, the water was drawn in by capillary forces.

d. Comparative example of capillary wetting

A piece of room temperature vulcanizing silicone rubber (Memosil, Wacker Chemie) having a microchannel pattern and two holes were placed onto the activated PET foil (lid) (according to a above). When a droplet of water was placed in the hole with a micropipette, no water was drawn in by capillary forces. When vacuum was applied to the channel through the other hole, the droplet was sucked into the channel.

CLAIMS

1. A microfluidic device comprising a set of one or more, preferably more than 5, covered microchannel structures manufactured in the surface of a planar substrate, **characterized** in that a part surface of at least one of the microchannel structures has a coat exposing a non-ionic hydrophilic polymer,that preferably is attached covalently directly to the surface or to a polymer skeleton that is attached to the surface.

2. The microfluidic device of claim 1, **characterized** in that the surface of the planar substrate is made of plastics.

3. The microfluidic device according to any of claims 1-2, **characterized** in that the non-ionic hydrophilic polymer is attached to the polymer skeleton that is attached to the part surface, said skeleton preferably being branched and/or preferably being a polyamine.

4. The microfluidic device according to any of claims 1-3, **characterized** in that the substrate surface without the coat is made of plastics and that said part surface without coat is hydrophilized by plasma treatment or by an oxidation agent in order to introduce functional groups that allow for a subsequent attachment of the coat onto said part surface.

5. The microfluidic device according to any of claims 1-4, **characterized** in that the non-ionic hydrophilic polymer comprises one or more blocks of polyoxyethylene chains, with preference for the polymer being polyethylene glycol covalently attached at one of its ends to the skeleton or directly to the part surface and possibly having the remaining hydroxy group etherified.

6. The microfluidic device according to any of claims 1-6, **characterized** in that the hydrophilic non-ionic polymer is a polyethylene glycol, preferably a monoalkoxy variant such as the monomethoxy variant, which is attached to said part surface via the polymer skeleton which preferably is a polyethylenimine.

7. The microfluidic device according to any of claims 1-6, **characterized** in that the hydrophilic non-ionic polymer is attached to said part surface or to said polymer skeleton via one-point attachment, preferably covalently.

8. The microfluidic device according to any of claims 2-7, **characterized** in that the plastics has a non-significant fluorescence for excitation wavelengths in the interval 200-800 nm and emission wavelengths in the interval 400-900 nm.

9. The microfluidic device according to any of claims 1-3 and 5-8, **characterized** in that said polymer skeleton is an inorganic or an organic polymer.

10. The microfluidic device according to any of claims 1-4 and 7-9, **characterized** in that said non-ionic hydrophilic polymer comprises a plurality of amide bonds, e.g. is polymerisate/copolymerisate with monomers at least selected from acrylamide, methacrylamide, vinylpyrrolidone etc.

11. The microfluidic device according to any of claims 1-10, **characterized** in that it is in a dried state that is capable of being rehydrated.

12. The use of the microfluidic device according to any of claims 1-11 in analytical systems in which an assay comprising one or more of the steps:
(a) sample preparation,

(b) assay reaction and

(c) detection,

at least one and preferably more than two of said steps being carried out within the microfluidic device.

INTERNATIONAL SEARCH REPORT

Inte ,onal Application No
PCT/EP 00/12478

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	EP 1 076 239 A (STUDIENGESELLSCHAFT KOHLE MBH) 14 February 2001 (2001-02-14) abstract; claims 1,7-17; figure 6 column 1, line 1 -column 1, line 18 column 4, line 26 -column 4, line 49 column 5, line 48 -column 6, line 4 column 6, line 33 -column 6, line 37	1-3,7, 11,12
A		4-6,8-10

X	DE 197 53 847 A (ROCHE DIAGNOSTICS GMBH) 10 June 1999 (1999-06-10) abstract; figure 1 column 3, line 67 -column 4, line 60 column 9, line 50 -column 10, line 33	1-3,7, 11,12
Y		4
A		5,6,8-10

	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search 16 March 2001	Date of mailing of the international search report 23/03/2001
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, **Fax: (+31-70) 340-3016**	Authorized officer Runser, C

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/EP 00/12478

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 99 58245 A (AMERSHAM PHARM BIOTECH AB ;ALLMER KLAS (SE); ANDERSSON PER (SE); L) 18 November 1999 (1999-11-18) cited in the application abstract; figures 6-8 page 2, line 7 -page 5, line 30 page 7, line 11 -page 8, line 25 page 10, line 8 -page 11, line 23	4
A		1-3,5-12
A	US 5 250 613 A (BERGSTROM KARIN ET AL) 5 October 1993 (1993-10-05) cited in the application the whole document	1-7,9,10
A	US 5 240 994 A (OSTERBERG EVA ET AL) 31 August 1993 (1993-08-31) cited in the application the whole document	1-7,9,10
A	US 5 858 188 A (SOANE DAVID S ET AL) 12 January 1999 (1999-01-12) cited in the application the whole document	1-12
A	EP 0 430 248 A (MOCHIDA PHARM CO LTD) 5 June 1991 (1991-06-05) abstract page 9, line 49 -page 10, line 12 page 10, line 33 -page 10, line 41	1,2,4,8, 11,12

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int .ional Application No
PCT/EP 00/12478

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1076239 A	14-02-2001	DE 19938002 A	15-02-2001
DE 19753847 A	10-06-1999	AU 2158399 A	28-06-1999
		CN 1284012 T	14-02-2001
		WO 9929429 A	17-06-1999
		EP 1035921 A	20-09-2000
WO 9958245 A	18-11-1999	AU 3624399 A	29-11-1999
		EP 1077771 A	28-02-2001
		GB 2350678 A	06-12-2000
		GB 2341924 A	29-03-2000
US 5250613 A	05-10-1993	SE 467308 B	29-06-1992
		AU 8747991 A	20-05-1992
		EP 0554318 A	11-08-1993
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		EP 0554324 A	11-08-1993
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		SE 9003364 A	23-04-1992
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US 5858188 A	12-01-1999	US 5126022 A	30-06-1992
		AU 715268 B	20-01-2000
		AU 2436497 A	29-10-1997
		CA 2249886 A	16-10-1997
		EP 0990147 A	05-04-2000
		JP 2000508763 T	11-07-2000
		WO 9738300 A	16-10-1997
		US 6054034 A	25-04-2000
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		AU 7467591 A	18-09-1991
		CA 2075969 A	29-08-1991
		EP 0521911 A	13-01-1993
		JP 3103031 B	23-10-2000
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		JP 2601595 B	16-04-1997
		JP 5504628 T	15-07-1993
		WO 9112904 A	05-09-1991
EP 0430248 A	05-06-1991	AU 642444 B	21-10-1993
		AU 6702690 A	06-06-1991
		CA 2031001 A	31-05-1991
		JP 3223674 A	02-10-1991
		US 5147607 A	15-09-1992

PATENT COOPERATION TREATY

ANKOM
2001 -11- 15

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

BERGANDER, Hakan
GYROS AB
Uppsala Science Park
75183 Uppsala
SUEDE

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)* 13.11.2001

REPLY DUE **within 3 month(s)** from the above date of mailing

Applicant's or agent's file reference GY 0024 PCT		
International application No. PCT/EP00/12478	International filing date *(day/month/year)* 11/12/2000	Priority date *(day/month/year)* 23/12/1999
International Patent Classification (IPC) or both national classification and IPC B01L3/00		
Applicant GYROS AB et al.		

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

   I ☒ Basis of the opinion
   II ☐ Priority
   III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   IV ☐ Lack of unity of invention
   V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   VI ☒ Certain document cited
   VII ☒ Certain defects in the international application
   VIII ☐ Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

   When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

   How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

   Also: For an additional opportunity to submit amendments, see Rule 66.4.
   For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
   For an informal communication with the examiner, see Rule 66.6.

   If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 23/04/2002.

Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer / Examiner Sembritzki, T Formalities officer (incl. extension of time limits) Fuerbass, C Telephone No. +49 89 2399 8132

WRITTEN OPINION

International application No. PCT/EP00/12478

I. Basis of the opinion

1. With regard to the **elements** of the international application (Replacement *sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed")*:

   Description, pages:

   1-21 as originally filed

   Claims, No.:

   1-12 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.
   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-12 : yes
Inventive step (IS)	Claims	1-12 : no
Industrial applicability (IA)	Claims	1-12 : yes

2. Citations and explanations
   see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

Reference is made to the following documents:

D1: EP-A-1 076 239 (STUDIENGESELLSCHAFT KOHLE MBH) 14 February 2001 (2001-02-14)
D2: DE 197 53 847 A (ROCHE DIAGNOSTICS GMBH) 10 June 1999 (1999-06-10)
D3: US-A-5 250 613 (BERGSTROM KARIN ET AL) 5 October 1993 (1993-10-05) cited in the application

Point V:

1. Novelty and inventive step

1.1 Document D3 discloses a non-ionic hydrophilic polymer which is coated on the surface of fluidic devices, such as biosensors or devices for affinity chromatography. The hydrophilic polymer is attached covalently to a polymer skeleton that is attached to the surface (see D3, column 1, line 8 - column 4, line 46). Similar coatings are already known for other micro fluidic devices as for example the interior walls of a capillary for capillary electrophoresis, this is correctly acknowledged on page 4, lines 19-30 of the description. Thus, the use of a non-ionic hydrophilic polymer coating is well known and commonly used in the field of micro fluidic devices. The only difference between the subject-matter of claim 1 of the present application and the prior art is the form of the micro fluidic device, i.e. a covered micro channel on a planar substrate, which is not explicitly disclosed in the above cited prior art documents.

However, also covered micro channel structures in the surface of planar substrates are well known from the prior art. Since non-ionic hydrophilic polymer coatings have already been employed for the same purpose in a similar devices, it would be obvious to the person skilled in the art, namely when the same result is to be achieved, to apply these features with known effect to such a micro channel structure thereby arriving at a device according to claim 1. The subject-matter of claim 1. does therefore not involve an inventive step (Article 33(3) PCT).

1.2 Furthermore, a covered micro channel structure in a micro fluidic device is known from document D2, which concerns a biosensor. In this document it is explicitly mentioned, that the surfaces should be hydrophilized using a coating of an inert hydrophilic polymer which is covalently bonded to the plastic surface of the channel (see D2, column 3, line 67 - column 4, line 60) so that a polar liquid is not prevented from being introduced into the micro channel. As can be seen in figure 3 of D2, only a part of the channel surface is provided with an hydrophilic layer. Even starting from this document as closest prior art, the subject-matter of claim 1 lacks inventive step, since a skilled person would use a non-ionic coating if influences on polar liquids should be avoided (Article 33(3) PCT).

1.3 If the problem to be solved with regard to D2 is only the selection of a suitable polymer, the skilled person would easily and without any inventive skill find the solution in document D3 which discloses the use of non-ionic hydrophilic coatings in biosensors, i.e. in the identical technical field. The subject-matter of claim 1 is therefore obvious also in view of a combination of D2 and D3 (Article 33(3) PCT).

1.4 From D2 it is known, that the planar substrate is made of plastic (claim 2), the biosensor of D2 serves as an analytical system wherein blood is prepared and brought to a reaction element where the presence and the amount of glucose is detected (see D2, examples) (claim 12). Accordingly, the subject-matter of claims 2 and 12 lacks inventive step (Article 33(2) PCT).

1.5 The subject-matter of the other dependent claims seems to contain only features which are either known from the prior art and which a skilled person could easily combine without any inventive skill, or which are the result of a normal design procedure followed by a skilled person (Article 33(3) PCT).

2. Industrial application

The industrial applicability is obvious.

Point VI:

Certain documents cited

Document D1 is published after the filing date of the present application but claims an older priority. This document could become relevant in the national or regional phase since its disclosure seems to be novelty destroying to the claims indicated in the search report.

Point VII:

Certain defects

The reference to "..is incorporated by reference.." (see page 8, line 17) should be removed (Rule 9(1iv) PCT).

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PATENT COOPERATION TREATY

REC'D 09 APR 2002
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference GY 0024 PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP00/12478	International filing date *(day/month/year)* 11/12/2000	Priority date *(day/month/year)* 23/12/1999
International Patent Classification (IPC) or national classification and IPC B01L3/00		
Applicant GYROS AB et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 9 sheets.

3. This report contains indications relating to the following items:

I ☒ Basis of the report
II ☐ Priority
III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV ☐ Lack of unity of invention
V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
VI ☐ Certain documents cited
VII ☐ Certain defects in the international application
VIII ☐ Certain observations on the international application

Date of submission of the demand 29/05/2001	Date of completion of this report 05.04.2002
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Sembritzki, T Telephone No. +49 89 2399 8626

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, pages:

1-4,7,9-21	as originally filed			
5,5a,6,8	as received on	08/02/2002	with letter of	06/02/2002

Claims, No.:

1-33	as received on	08/02/2002	with letter of	06/02/2002

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
☐ the language of publication of the international application (under Rule 48.3(b)).
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.
☐ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority in written form.
☐ furnished subsequently to this Authority in computer readable form.
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
   see separate sheet

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-12
Industrial applicability (IA)	Yes:	Claims	1-12
	No:	Claims	

2. Citations and explanations
   see separate sheet

Reference is made to the following documents:

D1: EP-A-1 076 239 (STUDIENGESELLSCHAFT KOHLE MBH) 14 February 2001 (2001-02-14)
D2: DE 197 53 847 A (ROCHE DIAGNOSTICS GMBH) 10 June 1999 (1999-06- 10)
D3: US-A-5 250 613 (BERGSTROM KARIN ET AL) 5 October 1993 (1993-10- 05) cited in the application

Point I:

1. According to Article 6 PCT the definition of the claims must be clear and understandable. The vague definition of a "microfluidic device" is nothing else but a small apparatus for handling fluids - neither an exact size nor a function, for example the transport of reagents is implicitly disclosed with such a definition. If the applicant is of the opinion that a "microfluidic device" represents something else, this has to be defined in the claims rather than in the description.

   All fluidic devices using capillary forces fall within the definition of a "microfluidic device", it is therefore not understood why capillaries used in capillary electrophoresis should be disclaimed if they are formed as covered microchannel structures. In this context is not clear, whether the disclaimer refers only to "sole capillaries" or to the use of the capillary device in capillary electrophoresis.
   The disclaimer is therefore not admissible.

   Furthermore, the disclaimer which was introduced into claim 1 only to differentiate the subject-matter of claim 1 from D1 (see **Point V**, 3. below) is senseless, since the disclosure of D1 is not limited to sole capillaries for capillary electrophoresis but refers to microfluidic structures and channels in general (see D1, claims 1-15 and 17).

2. The definition that "non-specific adsorption and hydrophilicity have been optimised by a coat..." is a mere result to be achieved, is a relative information and no technical feature (Article 6 PCT). The use of non-ionic hydrophilic polymers as coating in "microfluidic devices" is well known in the prior art, for example from

affinity chromatography or from biosensors (see D3 and **Point V**. 1.1 below). Since the coating as such is present, it cannot be differentiated whether the device is optimised for a special purpose or not. The "optimisation" in the present case relates to the use of the coating rather than to the coating as such.

3. The features of a non-ionic hydrophilic polymer coating as such on a microfluidic device comprising a covered microchannel manufactured in the surface of a planar substrate does not represent an inventive concept (see **Point V.** 1.1-1.3 below). The additional features of independent claims 1 and 31 define on the one hand the optimisation with regard to the adsorption and hydrophilicity (claim 1) and on the other hand the fluorescence of the plastic substrate (claim 31). It is obvious, that different problems should be solved and that the solution of both problems are not so linked as to form a single inventive concept (Rule 13.1 PCT). If the applicant is of the opinion that the optimisation is represented by the mere use of the non-ionic hydrophilic polymer coating, it is not understood, why a further independent claim is necessary. In this case claim 31 comprises all the features of claim 1 and is therefore not appropriately formulated as a claim dependent on the latter (Rule 6.4 PCT). If this interpretation would be valid, also D3 discloses a microfluidic device which is "optimised" with regard to adsorption and hydrophilicity even if this may not be explicitly mentioned.

4. The amendments of the description are inconsistent with regard to the numbering of the pages: Page 5 is followed by page 6 which is followed by page 5a. Amended page 5a should be followed by original page 6 so that two pages 6 would be existent. In this context the applicant's attention is drawn to the fact that, as a consequence of Rule 66.8(a) PCT the examiner is not permitted to carry out any amendments under the PCT procedure, however minor these may be.

5. Due to the points discussed above the amendments filed with letter of 06.02.2002 are not admissible. This report is therefore based on the application documents as originally filed.

Point V:

1. Novelty and inventive step

1.1 Document D3 discloses a non-ionic hydrophilic polymer which is coated on the surface of fluidic devices, such as biosensors or devices for affinity chromatography which both fall within the definition of a microfluidic device. The hydrophilic polymer is attached covalently to a polymer skeleton that is attached to the surface (see D3, column 1, line 8 - column 4, line 46). Similar coatings are already known for other micro fluidic devices as for example the interior walls of a capillary for capillary electrophoresis, this is correctly acknowledged on page 4, lines 19-30 of the description. Thus, the use of a non-ionic hydrophilic polymer coating is well known and commonly used in the field of micro fluidic devices. The only difference between the subject-matter of claim 1 of the present application and the prior art is the form of the micro fluidic device, i.e. a covered micro channel on a planar substrate, which is not explicitly disclosed in the above cited prior art documents.

However, also covered micro channel structures in the surface of planar substrates are well known from the prior art. Since non-ionic hydrophilic polymer coatings have already been employed for the same purpose in a similar devices, it would be obvious to the person skilled in the art, namely when the same result is to be achieved, to apply these features with known effect to such a micro channel structure thereby arriving at a device according to claim 1. The subject-matter of claim 1. does therefore not involve an inventive step (Article 33(3) PCT).

1.2 Furthermore, a covered micro channel structure in a micro fluidic device is known from document D2, which concerns a biosensor. In this document it is explicitly mentioned, that the surfaces should be hydrophilized using a coating of an inert hydrophilic polymer which is covalently bonded to the plastic surface of the channel (see D2, column 3, line 67 - column 4, line 60) so that a polar liquid is not prevented from being introduced into the micro channel.
As can be seen in figure 3 of D2, only a part of the channel surface is provided with an hydrophilic layer.

Even starting from this document as closest prior art, the subject-matter of claim 1 lacks inventive step, since a skilled person would use a non-ionic coating if influences on polar liquids should be avoided (Article 33(3) PCT).

1.3 If the problem to be solved with regard to D2 is only the selection of a suitable polymer, the skilled person would easily and without any inventive skill find the solution in document D3 which discloses the use of non-ionic hydrophilic coatings in biosensors which represent microfluidic devices, i.e. in the identical technical field. The subject-matter of claim 1 is therefore obvious also in view of a combination of D2 and D3 (Article 33(3) PCT).

1.4 From D2 it is known, that the planar substrate is made of plastic (claim 2), the biosensor of D2 serves as an analytical system wherein blood is prepared and brought to a reaction element where the presence and the amount of glucose is detected (see D2, examples) (claim 12). Accordingly, the subject-matter of claims 2 and 12 lacks inventive step (Article 33(2) PCT).

1.5 The subject-matter of the other dependent claims seems to contain only features which are either known from the prior art and which a skilled person could easily combine without any inventive skill, or which are the result of a normal design procedure followed by a skilled person (Article 33(3) PCT).

2. Industrial application

The industrial applicability is obvious.

3. Certain documents cited

Document D1 is published after the filing date of the present application but claims an older priority. This document could become relevant in the national or regional phase since its disclosure seems to be novelty destroying to the claims indicated in the search report.

4. Certain defects

The reference to "..is incorporated by reference.." (see page 8, line 17) should be removed (Rule 9(1iv) PCT).

least one of the substrates. For aqueous liquids the hydrophilic areas define the fluid pathways. Various ways of obtaining a pattern of hydrophobic and hydrophilic surfaces for different purposes are discussed, for instance, plasma treatment, coating a hydrophobic surfaces with a hydrophilic polymer etc. The hydrophilic coat polymers suggested may or may not have aryl groups suggesting that Larsson et al are not focusing on lowering the water contact angle as much as possible or avoiding non-specific adsorption.

Larsson, Ocklind and Derand (WO 0056808) describe the production of highly hydrophilic surfaces made of plastics. The surfaces retain their hydrophilicity even after being in contact with aqueous liquids. An additional issue in WO 0056808 is to balance a permanent hydrophilicity with good cell attachment properties. The surfaces are primarily suggested to be used in microfabricated devices.

Polyethylene glycol has been linked directly to the surface of a microchannel fabricated in silicone for testing the ability of polyethylyne glycol to prevent protein adsorption. See Bell, Brody and Yager (SPIE-Int. Soc. Opt. Eng. (1998) 3258 (Micro- and Nanofabricated Structures and Devices for Biomedical Environmental Applications) 134-140).

One of the inventors (James Van Alstine) has in a series of articles described how various coats of non-ionic hydrophilic polymers on hydrophilic materials, such as polyethylene glycol either alone or as polyethylene imine polyethylene glycol adducts, reduce electroosmosis, non-specific adsorption of proteins and influence wettability in a microchannel. See Burns et al (Langmuir 11 (1995) 2768-2776, Emoto et al (ACS Symp. Ser 680 (Poly(ethylene glycol) (1997) 374-299, Van Alstine et al (Colloids and Surfaces B: Biointerfaces 14 (1999) 197-211), Knox et al (Anal. Chem. 70 (1998) 2268-2279;

materials involved already exhibited suitable wetting. What was noteworthy to the authors at that time was that the PEG coatings did not negatively affect the wettability.

AU 9921583 (= DE 19753847.9)(Zimmer et al) describes a test device comprising a microchannel which is communicating with an inlet and a detector zone. Liquid transport is solely dependent on capillary force. The surfaces of the microchannel can expose a surface coat of a hydrophilic polymer to promote capillary transport of an aqueous sample. Device materials with pronounced non-specific adsorption are suggested.

The objectives of the invention.

A first objective is to accomplish a sufficiently reliable and reproducible mass transport of reagents and sample constituents (e.g. analytes) in microfluidic devices.

A second objective is to enable a reliable and reproducible aqueous liquid flow in the microfluidic devices.

Malmsten et al (J. Coll. Interface Sci. 202 (1998) 507-517). The goal of this work was not to favourably control wettability (hydrophilicity) since the quartz and other

polymerisation of epichlorohydrin, glycidol and similar bifunctionally reactive monomers giving polyhydroxy polymers.

Polyvinylpyrrolidone (PVP), polyacrylamides, polymethacrylamides etc are examples of polymers in which there are a plurality of amide groups.

Further suitable hydrophilic polymers are reaction products (adducts) between ethylene oxide, optionally in combination with higher alkylene oxides or bisepoxides, or tetrahydrofuran, and a dihydroxy or polyhydroxy compound as illustrated with glycerol, pentaerythritol and any of the polyhydroxy polymers referred to in the preceding paragraphs.

The non-ionic hydrophilic polymer may have the same structure as described for the extenders defined in Berg et al (WO 9833572). In contrast to Berg et al there is no imperative need for the presence of an affinity ligand on the hydrophilic polymer used in the present invention.

One or more positions in the non-ionic hydrophilic polymer may be utilized for attachment. In order to make the hydrophilic polymer flexible the number of attachment points should be as low as possible, for instance one, two or three positions per polymer molecule. For straight chain polymers, such as lower alkylene oxide polymers similar to polyethylene oxide, the number of attachment points is typically one or two, with preference for one.

Depending on the position of a coated part surface within a microchannel structure, the hydrophilic polymer may carry an immobilized reactant (often called ligand when affinity reactions are concerned). Depending on the particular use of a microchannel structure such reactants can be so called

CLAIMS

1. A microfluidic device comprising a set of one or more covered microchannel structures manufactured in the surface of a planar substrate, with the proviso that sole capillaries, possibly with an area for application and an area for detection, as used in capillary electrophoresis are excluded from being a microfluidic device, **characterized** in that non-specific adsorption and hydrophilicity have been optimised by a coat exposing a non-ionic hydrophilic polymer on a part of the surface of at least one of the microchannel structures.

2. The microfluidic device of claim 1, **characterized** in that the surface carrying the coat is made of organic or inorganic material.

3. The microfluidic device of any of claims 1-2, **characterized** in that the surface of the planar substrate is made of plastics.

4. The microfluidic device of any of claims 1-3, **characterized** in that non-ionic hydrophilic polymer is attached covalently directly to the surface or to a polymer skeleton that is attached to the surface.

5. The microfluidic device of any one of claims 1-4, **characterized** in that there are more than five covered microchannel structures.

6. The microfluidic device of any of claims 1-5, **characterized** in that each microchannel structures comprises a functional part selected amongst (a) application chamber or cavity, (b) conduit for liquid transport, (c) reaction chamber or cavity, (d) volume defining unit, (e) mixing chamber or cavity, (f) chamber for separating components of the sample, (g) detection chamber or cavity.

7. The microfluidic device of any of claims 1-6, **characterized** in that the non-ionic hydrophilic polymer is present on the surface of at least one of said functional parts and gives the surface a sufficient hydrophilicity for liquid to enter the part once having passed the entrance of the part.

8. The microfluidic device of any of claims 1-7, **characterized** in that each microchannel structure comprises a microcavity having a volume ≤ 1 µl.

9. The microfluidic device of any of claims 1-8, **characterized** in that mass transport of solutes and/or particles between different functional parts of each microchannel structure uses a liquid flow driven by a) non-electrokinetic forces and/or b) electroendoosmosis.

10. The microfluidic device of any of claims 1-9, **characterized** in that the device is a round disc and that the liquid flow is driven by capillary action, centripetal force (spinning the disc) and/or hydrodynamically.

11. The microfluidic device of any of claims 1-10, **characterized** in that the non-ionic hydrophilic polymer is selected amongst polymers containing a plurality of hydroxy groups, ethylene oxy groups, amide groups.

12. The microfluidic device of claim 11, **characterized** in that the non-ionic hydrophilic polymer is a polyhydroxy polymer.

13. The microfluidic device of claim 11, **characterized** in that the non-ionic hydrophilic polymer is selected amongst polysaccharides and water-soluble derivatives thereof, polyvinyl alcohols, poly(hydroxy alkyl vinylether) polymers.

14. The microfluidic device of claim 11, **characterized** in that non-ionic hydrophilic polymer is a reaction product between ethylene oxide and a dihydroxy or a polyhydroxy compound.

15. The microfluidic device of claim 11, characterized in that the non-ionic hydrophilic polymer comprises one or more blocks of polyoxyethylene chains.

16. The microfluidic device of claim 11, **characterized** in that the non-ionic hydrophilic polymer is polyethylene glycol.

17. The microfluidic device of claim 11, characterized in that the non-ionic hydrophilic polymer is polyethylene glycol which has a methoxy group at the end which does not bind directly to the part surface or to the skeleton, if present.

18. The microfluidic device of claim 11, **characterized** in that the non-ionic hydrophilic polymer comprises a plurality of amide groups.

19. The microfluidic device of claim 11, **characterized** in that the non-hydrophilic polymer a polymerisate/co-polymerisate with monomers selected from at least acrylamide, methacrylamide and vinylpyrrolidone.

20. The microfluidic device according to any of claims 1-20, **characterized** in that the non-ionic hydrophilic polymer is attached to a polymer skeleton that is attached to the part surface.

21. The microfluidic device of claim 20 **characterized** in that said attachment between the non-ionic hydrophilic polymer and the polymer skeleton is covalent.

22. The microfluidic device of claim 21, **characterized** in that said polymer skeleton is an inorganic or an organic polymer.

23. The microfluidic device of any of claims 20-22, **characterized** in that said skeleton is selected among cationic, anionic, and neutral polymers.

24. The microfluidic device of any of claims 20-23, **characterized** in that said skeleton is selected among polymers that are polyamines.

25. The microfluidic device of any of claims 20-24, **characterized** in that said skeleton is a polyethylene imine.

26. The microfluidic device of any of claims 20-25, **characterized** in that the skeleton has a molecular weight 10,000-3,000,000 dalton.

27. The microfluidic device according any of claims 1-26, **characterized** in that the substrate surface without the coat is made of plastics and that said part surface without coat is hydrophilized by plasma treatment or by an oxidation agent in order to introduce functional groups that allow for a subsequent attachment of the coat onto said part surface.

28. The microfluidic device according to any of claims 1-27, **characterized** in that in that the surface of the planar substrate is made of plastics and that the plastics has a non-significant fluorescence for excitation wavelengths in the interval 200-800 nm and emission wavelengths in the interval 400-900 nm.

29. The microfluidic device according to any of claims 1-28, **characterized** in that it is in a dried state that is capable of being rehydrated.

30. The use of the microfluidic device of any of claims 1-29 in analytical systems in which an assay comprising one or more of the steps of:

sample preparation,
assay reaction and
detection,

at least one and preferably more than two of said steps being carried out within the microfluidic device.

31. A microfluidic device comprising a set of one or more, preferably more than 5, covered microchannel structures manufactured in the surface of a planar substrate, **characterized** in that a part surface of at least one of the microchannel structures has a coat exposing a non-ionic hydrophilic polymer and that the surface of the planar substrate is made of plastics that has a non-significant fluorescence for excitation wavelengths in the interval 200-800 nm and emission wavelengths in the interval 400-900 nm.

32. The microfluidic device of claim 31, **characterized** in that the plastics is based on a polymer of aliphatic monomers containing polymerizable carbon-carbon double bonds.

33. The microfluidic device of claim 33, **characterized** in that the monomer is selected among is a cycloalkane, norbornene or substituted norbornene, ethylene and propylene.

Copy for the Elected Office (EO/US) PCT EP00 1247

P ENT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

To:

BERGANDER, Hakan
Gyros AB
Uppsala Science Park
SE-751 83 Uppsala
SUÈDE

Date of mailing (day/month/year) 14 August 2001 (14.08.01)	
Applicant's or agent's file reference GY 0024 PCT	**IMPORTANT NOTIFICATION**
International application No. PCT/EP00/12478	**International filing date** (day/month/year) 11 December 2000 (11.12.00)

1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

Name and Address ROLLINS, Anthony, John Nycomed Amersham plc Amersham Laboratories White Lion Road Amersham, Buckinghamshire HP7 9LL United Kingdom	State of Nationality	State of Residence
	Telephone No. +44 1494 543090	
	Facsimile No. +44 1494 543977	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☒ the person ☐ the name ☐ the address ☐ the nationality ☐ the residence

Name and Address BERGANDER, Hakan Gyros AB Uppsala Science Park SE-751 83 Uppsala Sweden	State of Nationality	State of Residence
	Telephone No. 46 18 566361	
	Facsimile No. 46 18 566350	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office
☐ the International Searching Authority
☒ the International Preliminary Examining Authority
☐ the designated Offices concerned
☒ the elected Offices concerned
☐ other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer R. Raissi Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2 5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 14 August 2001 (14.08.01)	
International application No. PCT/EP00/12478	**Applicant's or agent's file reference** GY 0024 PCT
International filing date (day/month/year) 11 December 2000 (11.12.00)	**Priority date** (day/month/year) 23 December 1999 (23.12.99)
Applicant DERAND, Helene et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

29 May 2001 (29.05.01)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was

☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

R. Raissi

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PL9968-PCT	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/EP 00/ 12478	International filing date *(day/month/year)* 11/12/2000	(Earliest) Priority Date *(day/month/year)* 23/12/1999
Applicant GYROS AB		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readble form.
   ☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).
3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

   ☐ as suggested by the applicant.
   ☐ because the applicant failed to suggest a figure.
   ☐ because this figure better characterizes the invention.

   ☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/12478

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
E	EP 1 076 239 A (STUDIENGESELLSCHAFT KOHLE MBH) 14 February 2001 (2001-02-14) abstract; claims 1,7-17; figure 6 column 1, line 1 -column 1, line 18 column 4, line 26 -column 4, line 49 column 5, line 48 -column 6, line 4 column 6, line 33 -column 6, line 37	1-3,7, 11,12
A		4-6,8-10

X	DE 197 53 847 A (ROCHE DIAGNOSTICS GMBH) 10 June 1999 (1999-06-10) abstract; figure 1 column 3, line 67 -column 4, line 60 column 9, line 50 -column 10, line 33	1-3,7, 11,12
Y		4
A		5,6,8-10

	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 16 March 2001	Date of mailing of the international search report 23/03/2001
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Runser, C

International Application No
PCT/EP 00/12478

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	WO 99 58245 A (AMERSHAM PHARM BIOTECH AB ;ALLMER KLAS (SE); ANDERSSON PER (SE); L) 18 November 1999 (1999-11-18) cited in the application abstract; figures 6-8 page 2, line 7 -page 5, line 30 page 7, line 11 -page 8, line 25 page 10, line 8 -page 11, line 23	4
A		1-3,5-12
A	US 5 250 613 A (BERGSTROM KARIN ET AL) 5 October 1993 (1993-10-05) cited in the application the whole document	1-7,9,10
A	US 5 240 994 A (OSTERBERG EVA ET AL) 31 August 1993 (1993-08-31) cited in the application the whole document	1-7,9,10
A	US 5 858 188 A (SOANE DAVID S ET AL) 12 January 1999 (1999-01-12) cited in the application the whole document	1-12
A	EP 0 430 248 A (MOCHIDA PHARM CO LTD) 5 June 1991 (1991-06-05) abstract page 9, line 49 -page 10, line 12 page 10, line 33 -page 10, line 41	1,2,4,8, 11,12

INTER[illegible]TIONAL SEARCH REPORT

Information on patent family members

In[illegible]onal Application No
PCT/EP 00/12478

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1076239 A	14-02-2001	DE 19938002 A	15-02-2001
DE 19753847 A	10-06-1999	AU 2158399 A	28-06-1999
		CN 1284012 T	14-02-2001
		WO 9929429 A	17-06-1999
		EP 1035921 A	20-09-2000
WO 9958245 A	18-11-1999	AU 3624399 A	29-11-1999
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		GB 2341924 A	29-03-2000
US 5250613 A	05-10-1993	SE 467308 B	29-06-1992
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		EP 0554318 A	11-08-1993
		JP 6502156 T	10-03-1994
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		SE 9003364 A	23-04-1992
		WO 9207023 A	30-04-1992
US 5858188 A	12-01-1999	US 5126022 A	30-06-1992
		AU 715268 B	20-01-2000
		AU 2436497 A	29-10-1997
		CA 2249886 A	16-10-1997
		EP 0990147 A	05-04-2000
		JP 2000508763 T	11-07-2000
		WO 9738300 A	16-10-1997
		US 6054034 A	25-04-2000
		US 5750015 A	12-05-1998
		US 6093296 A	25-07-2000
		AU 637895 B	10-06-1993
		AU 7467591 A	18-09-1991
		CA 2075969 A	29-08-1991
		EP 0521911 A	13-01-1993
		JP 3103031 B	23-10-2000
		JP 8327597 A	13-12-1996
		JP 2601595 B	16-04-1997
		JP 5504628 T	15-07-1993
		WO 9112904 A	05-09-1991
EP 0430248 A	05-06-1991	AU 642444 B	21-10-1993
		AU 6702690 A	06-06-1991
		CA 2031001 A	31-05-1991
		JP 3223674 A	02-10-1991
		US 5147607 A	15-09-1992

10/069827

JC Rec'd PCT/PTO 26 FEB 2002

European Patent Office
Erhardtstrasse 27
D-802 98 Munich
Germany

Uppsala, February 6, 2002

Dear Sirs;

Re. International patent application PCT/EP00/12478.
Our ref. GY 0024 PCT.

This is in response to the Written Opinion of November 13, 2001.

Amended pages.
Amended pages 5, 5a and page 8 are attached in triplicate and shall replace pages 5 and 8.

On page 5, lines 11-12 and 15 the WO publication number has replaced the application number.

On page 5a, articles authored or co-authored by one of the present inventors (James Van Alstine) and D2 have been added and commented. The comments are self-explanatory.

On page 8, "is incorporated by reference" has been removed as suggested in the Written Opinion Point VII. We reserve the right to re-enter the phrase for the US national phase.

The amendments have been indicated on separate pages.

Amended claims
A new set of claims is attached in triplicate (pages 22-26). The support in the original application for the new set of claims is given in blue on a separate set of claims.

New claim 1 has been clarified in that the coat exposing the non-ionic hydrophilic polymer optimizes hydrophilicity and non-specific adsorption in relation to each other. Support is on page 6, lines 1-3. A disclaimer relative to sole capillary for capillary electrophoresis has also been inserted. This is in line with the definition for microfluidic device given on page 1-2, bridging paragraph.

We have included an alternative set of independent claim (claim 31-33). Claim 31 is a combination of original claims 1 and 8.

We reserve the right to take up parts that have been excluded by the new set of claims in forth-coming national proceedings.

Point V of the Written Opinion.

1. Novelty and Inventive step

1.1. The Written Opinion says that document D3 (US 5,250,613, Bergström et al) describes a fluidic device which utilizes a non-ionic hydrophilic polymer coating, and that our specification on page 4, lines 19-30, says that similar coatings as in claim 1 have been used in capillary electrophoresis. The Written Opinion then concludes that the only difference between the prior art and our claim 1 is the form of the microfluidic device, i.e. a covered microchannel on a planar substrate.

This argument is incorrect and based on a simplified view from the macroworld. It does not account for the particular problems that have to be solved when downscaling within the microworld.

Counter argument
The problem to be solved is to balance non-specific adsorption of reagents and/or the analyte with the hydrophilicity of the microchannels while securing a reliable and reproducible mass transport by a liquid flow between different functional parts of a microfluidic devices. See the definition of microfluiduic device (pages 2-3, bridging paragraph), page 2, line 17, to page 4, line 3, and under the heading "Objectives of the Invention" in our application text. A good hydrophilicity is required in order to facilitate liquid flow. A low non-specific adsorption is required for the reactions to take place properly. It is not as simple that an increased hydrophilicity also means a decreased non-specific adsorption. The balancing becomes more and more difficult when dimensions are going down into the microworld. The most important reason is that the problem with non-specific adsorption increases with the surface to volume ratio. Compare page 3, lines 13-22, of our application text, and our comment about aluminum oxide under point 1.2.

This is illustrated on the attached sales material from Gyros AB (page 8 in "The Technology Platform), which illustrates that without coating according to the invention enzyme activity may be significantly reduced in a microchamber due to non-specific adsorption. The volume of the chamber in figure 14 is $\leq 1\ \mu l$.

Claim 8 has been added in order to stress the importance of down scaling when determining inventive step. This claims says that each microchannel structure comprises a microcavity having a volume $\leq 1\ \mu l$.

D 3 says nothing about fluidic devices. There is nothing about transport of reagents by a liquid flow.

The publications discussed on page 4, lines 19-30, of the originally filed application refer to capillary electrophoresis for the separation of solutes. However, these publications focus on the control and/or reduction of electroosmosis by providing a surface coat that consists of a hydrophilic polymer. The reason for avoiding uncontrolled electroosmosis is that it disturbs the efficiency of capillary electrophoresis. This has nothing to do with transporting reagents in a microfluidic device by a liquid flow. To stress this we have inserted a disclaimer for capillary electrophoresis into claim 1.

None of the publications referred to in the Written Opinion under this Point takes up the problem the present invention sets out to solve. Nor is a solution to the problem given. This is strong argument for inventive step. The Written Opinion is therefore incorrect when concluding lack of inventive step in paragraph 1.1.

1.2. In this paragraph the Written Opinion argues lack of inventive step over Document D2. This document is said to describe a microfluidic device that is a biosensor.

D2 (DE 19753847 = AU 21583/99) describes a device which has a single microchannel starting at a sample inlet and communicating with a detection zone. Liquid transport within the device is based on capillarity. D2 therefore stresses that it is important to hydrophilize the walls of the microchannel if they are not sufficiently hydrophilic by themselves. The problem about non-specific adsorption is not dealt with. In fact D2 suggests capillary surfaces that expose oxidized aluminum in preferred variants. See pages 8-9, bridging sentence and example 1 (Australian counterpart). This kind of surfaces is likely to give pronounced adsorption of proteins (i.e. pronounced non-specific adsorption). This in turn strongly suggests that the inventors of D2 didn't recognize the problem with non-specific adsorption. The reason might be that in the actual work done (example 1), the dimensions were relatively large (channel length 15 mm, width 2 mm and height 0.1 mm, and volume 3 μl). It may also depend on the particular assay that was used.

Our microfluidic device is based on the recognition that it becomes utterly important and intricate to balance hydrophilicity and non-specific adsorption when downscaling within the microworld. The fact that D2 is silent about the problem and its solution strongly supports inventive step.

The Australian counter part (AU 21583/99) to D2 is enclosed.

1.3. The Written Opinion suggests that, if the problem to be solved with regard to D2 is only the selection of a suitable polymer, then there is no inventive step because D3 discloses the use of a non-ionic hydrophilic coat in biosensors, i.e. in the same field as D2.

This argument is incorrect for a number of reasons:

- The present invention belongs to the field defined in the preamble of claim 1, i.e. microfluidic devices. According to the definition in our application text this means that there has to be "a liquid flow that causes mass transport of solutes and/or particles dispersed in the liquid from one functional part of the structure to

another". See pages 1-2, bridging sentence. This is not the definition of a biosensor. The discussion about biosensor in the Written Opinion is therefore irrelevant.

- The device of D2 is not a biosensor. This means that it is incorrect to say that D2 and D3 belong to the same technical field because they deal with biosensors.
- D3 is silent about what kind of biosensor is intended. Therefore it is incorrect to link the statement about biosensor in D3 to a microfluidic device.

The present invention has inventive step in view of the combination D2 and D3. One of the main reasons is that D2 says nothing about the problem the present invention sets out to solve. Therefore there is no reason to use the particular hydrophilic polymers of D3 and apply them as a surface coat in the microchannel of D2 in order to reach to the invention.

1.4. In this paragraph the Written Opinion argues lack of inventive step for original claim 2 and original claim 12 in view of the teachings of D2. This is not a correct argument for the same reasons as discussed for claim 1. In addition one has to bear in mind that it is much more difficult to arrange for the proper balance between surface hydrophilicity and non-specific adsorption for a reliable and reproducibly mass transport of reagents and analytes in plastics than in hydrophilic materials. See page 3, lines 24-29, of our original application text. This supports inventive step of claim 2 over claim 1.

1.5. This paragraph argues lack of inventive step for all other claims. This argument is incorrect. These subclaims have inventive step because claim 1 has inventive step as discussed above but also for several other reasons. We decline from discussing these other reasons at the present stage and reserve the right to come back to this issue in the national phase proceedings.

Point VI of the Written Opinion.

Document D1 (EP 1076239) concerns sole capillaries for capillary electrophoresis. This kind of capillaries is not microfluidic devices. In order to avoid this kind of misunderstanding new claim 1 explicitly disclaims electrophoretic capillaries.

Based on the comments given above we are expecting an International Examination Report that is positive for claims 1-30. We decline from arguing patentability for claims 31-33.

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Enclosures:

- Amended pages 5,5a, and 8 (3x)
- Amended pages 5, 5a, 8 with amendments indicated (1x)
- New set of claims pages 22-26 (3x)
- New set of claims with support for the individual claims in blue. Pages 22-27 (1x)
- AU 9921583 (= D2 = DE 19753847.9)
- Burns et al., Langmuir 11 (1995) 2768-2776
- Emoto et al., ACS Symp. Ser 680 (Poly(ethylene glycol) (1997) 374-299
- Van Alstine et al., Colloids and Surfaces B: Biointerfaces 14 (1999) 197-211
- Knox et al., Anal. Chem. 70 (1998) 2268-2279
- Malmsten et al., J. Coll. Interface Sci. 202 (1998) 507-517
- The Technology Platform (Gyros AB), front page and page 8
- Copy of G.A. 43194
- Form 1037

C L A I M S

1. A microfluidic device comprising a set of one or more covered microchannel structures manufactured in the surface of a planar substrate, with the proviso that sole capillaries, possibly with an area for application and an area for detection, as used in capillary electrophoresis are excluded from being a microfluidic device, **characterized** in that non-specific adsorption and hydrophilicity have been optimised by a coat exposing a non-ionic hydrophilic polymer on a part of the surface of at least one of the microchannel structures. Support on page 2, lines 1-3 (disclaimer) and page 6, lines 1-3.

2. The microfluidic device of claim 1, **characterized** in that the surface carrying the coat is made of organic or inorganic material. Page 14, lines 30-31.

3. The microfluidic device of any of claims 1-2, **characterized** in that the surface of the planar substrate is made of plastics. Original claim 2.

4. The microfluidic device of any of claims 1-3, **characterized** in that non-ionic hydrophilic polymer is attached covalently directly to the surface or to a polymer skeleton that is attached to the surface. Original claim 1.

5. The microfluidic device of any one of claims 1-4, **characterized** in that there are more than five covered microchannel structures. Original claim 1.

6. The microfluidic device of any of claims 1-5, **characterized** in that each microchannel structures comprises a functional part selected amongst (a) application chamber or cavity, (b) conduit for liquid transport, (c) reaction chamber or cavity, (d) volume defining unit, (e) mixing chamber or cavity, (f) chamber

for separating components of the sample, (g) detection chamber or cavity. Page 12, lines 24-33.

7. The microfluidic device of any of claims 1-6, **characterized** in that the non-ionic hydrophilic polymer is present on the surface of at least one of said functional parts and gives the surface a sufficient hydrophilicity for liquid to enter the part once having passed the entrance of the part. Page 2, lines 29-34 and pages 12-13, bridging sentence.

8. The microfluidic device of any of claims 1-7, **characterized** in that each microchannel structure comprises a microcavity having a volume ≤ 1 μl. Page 14, line 12.

9. The microfluidic device of any of claims 1-8, **characterized** in that mass transport of solutes and/or particles between different functional parts of each microchannel structure uses a liquid flow driven by a) non-electrokinetic forces and/or b) electroendoosmosis. Page 13, lines 8-14.

10. The microfluidic device of any of claims 1-9, **characterized** in that the device is a round disc and that the liquid flow is driven by capillary action, centripetal force (spinning the disc) and/or hydrodynamically. Page 12, line 17-22.

11. The microfluidic device of any of claims 1-10, **characterized** in that the non-ionic hydrophilic polymer is selected amongst polymers containing a plurality of hydroxy groups, ethylene oxy groups, amide groups. Page 6, lines 28-32.

12. The microfluidic device of claim 11, **characterized** in that the non-ionic hydrophilic polymer is a polyhydroxy polymer. Page 7, lines 20-22.

13. The microfluidic device of claim 11, **characterized** in that the non-ionic hydrophilic polymer is selected amongst polysaccharides and water-soluble derivatives thereof, polyvinyl alcohols, poly(hydroxy alkyl vinylether) polymers. Page 7, lines 32-34.

14. The microfluidic device of claim 11, **characterized** in that non-ionic hydrophilic polymer is a reaction product between ethylene oxide and a dihydroxy or a polyhydroxy compound. Page 8, lines 8-13.

15. The microfluidic device of claim 11, characterized in that the non-ionic hydrophilic polymer comprises one or more blocks of polyoxyethylene chains. Original claim 5.

16. The microfluidic device of claim 11, **characterized** in that the non-ionic hydrophilic polymer is polyethylene glycol. Page 7, lines 7-8 and original claim 5.

17. The microfluidic device of claim 11, characterized in that the non-ionic hydrophilic polymer is polyethylene glycol which has a methoxy group at the end which does not bind directly to the part surface or to the skeleton, if present. Original claim 6.

18. The microfluidic device of claim 11, **characterized** in that the non-ionic hydrophilic polymer comprises a plurality of amide groups. Original claim 10.

19. The microfluidic device of claim 11, **characterized** in that the non-hydrophilic polymer a polymerisate/copolymerisate with monomers selected from at least acrylamide, methacrylamide and vinylpyrrolidone. Original claim 10.

20. The microfluidic device according to any of claims 1-20, **characterized** in that the non-ionic hydrophilic

polymer is attached to a polymer skeleton that is attached to the part surface. Original claim 3.

21. The microfluidic device of claim 20 **characterized** in that said attachment between the non-ionic hydrophilic polymer and the polymer skeleton is covalent. Original claim 7.

22. The microfluidic device of claim 21, **characterized** in that said polymer skeleton is an inorganic or an organic polymer. Original claim 9.

23. The microfluidic device of any of claims 20-22, **characterized** in that said skeleton is selected among cationic, anionic, and neutral polymers. Page 9, lines 8-9.

24. The microfluidic device of any of claims 20-23, **characterized** in that said skeleton is selected among polymers that are polyamines. Page 9, lines 14-15, and original claim 3.

25. The microfluidic device of any of claims 20-24, **characterized** in that said skeleton is a polyethylene imine. Page 9, line 34.

26. The microfluidic device of any of claims 20-25, **characterized** in that the skeleton has a molecular weight 10,000-3,000,000 dalton. Page 9, line 31.

27. The microfluidic device according any of claims 1-26, **characterized** in that the substrate surface without the coat is made of plastics and that said part surface without coat is hydrophilized by plasma treatment or by an oxidation agent in order to introduce functional groups that allow for a subsequent attachment of the coat onto said part surface. Original claim 4.

28. The microfluidic device according to any of claims 1-27, **characterized** in that in that the surface of the planar substrate is made of plastics and that the plastics has a non-significant fluorescence for excitation wavelengths in the interval 200-800 nm and emission wavelengths in the interval 400-900 nm. Original claim 8.

29. The microfluidic device according to any of claims 1-28, **characterized** in that it is in a dried state that is capable of being rehydrated. Original claim 11.

30. The use of the microfluidic device of any of claims 1-29 in analytical systems in which an assay comprising one or more of the steps of:

sample preparation,
assay reaction and
detection,

at least one and preferably more than two of said steps being carried out within the microfluidic device. Original claim 11.

31. A microfluidic device comprising a set of one or more, preferably more than 5, covered microchannel structures manufactured in the surface of a planar substrate, **characterized** in that a part surface of at least one of the microchannel structures has a coat exposing a non-ionic hydrophilic polymer and that the surface of the planar substrate is made of plastics that has a non-significant fluorescence for excitation wavelengths in the interval 200-800 nm and emission wavelengths in the interval 400-900 nm. Original claim 1 combined with original claim 8.

32. The microfluidic device of claim 31, **characterized** in that the plastics is based on a polymer of aliphatic monomers containing polymerizable carbon-carbon double bonds. Page 16, lines 20-25.

33. The microfluidic device of claim 33, **characterized** in that the monomer is selected among is a cycloalkane, norbornene or substituted norbornene, ethylene and propylene. Page 16, lines 20-25.

least one of the substrates. For aqueous liquids the hydrophilic areas define the fluid pathways. Various ways of obtaining a pattern of hydrophobic and hydrophilic surfaces for different purposes are discussed, for instance, plasma treatment, coating a hydrophobic surfaces with a hydrophilic polymer etc. The hydrophilic coat polymers suggested may or may not have aryl groups suggesting that Larsson et al are not focusing on lowering the water contact angle as much as possible or avoiding non-specific adsorption.

Larsson, Ocklind and Derand (WO 0056808~~PCT/EP00/05193 claiming priority from SE 9901100-9, filed 1999-03-24~~) describe the production of highly hydrophilic surfaces made of plastics. The surfaces retain their hydrophilicity even after being in contact with aqueous liquids. An additional issue in ~~PCT/EP00/05193~~WO 0056808 is to balance a permanent hydrophilicity with good cell attachment properties. The surfaces are primarily suggested to be used in microfabricated devices.

Polyethylene glycol has been linked directly to the surface of a microchannel fabricated in silicone for testing the ability of polyethylyne glycol to prevent protein adsorption. See Bell, Brody and Yager (SPIE-Int. Soc. Opt. Eng. (1998) 3258 (Micro- and Nanofabricated Structures and Devices for Biomedical Environmental Applications) 134-140).

One of the inventors (James Van Alstine) has in a series of articles described how various coats of non-ionic hydrophilic polymers on hydrophilic materials, such as polyethylene glycol either alone or as polyethylene imine polyethylene glycol adducts, reduce electroosmosis, non-specific adsorption of proteins and influence wettability in a microchannel. See Burns et al (Langmuir 11 (1995) 2768-2776, Emoto et al (ACS Symp. Ser 680 (Poly(ethylene glycol) (1997) 374-299, Van Alstine et al (Colloids and Surfaces B: Biointerfaces 14 (1999) 197-211), Knox et al (Anal. Chem. 70 (1998) 2268-2279; Malmsten et al (J. Coll. Interface Sci. 202 (1998) 507-517). The goal of this work was not to favourably control wettability (hydrophilicity) since the quartz and other

materials involved already exhibited suitable wetting. What was noteworthy to the authors at that time was that the PEG coatings did not negatively affect the wettability.

AU 9921583 (= DE 19753847.9) (Zimmer et al) describes a test device comprising a microchannel which is communicating with an inlet and a detector zone. Liquid transport is solely dependent on capillary force. The surfaces of the microchannel can expose a surface coat of a hydrophilic polymer to promote capillary transport of an aqueous sample. Device materials with pronounced non-specific adsorption are suggested.

The objectives of the invention.

A first objective is to accomplish a sufficiently reliable and reproducible mass transport of reagents and sample constituents (e.g. analytes) in microfluidic devices.

A second objective is to enable a reliable and reproducible aqueous liquid flow in the microfluidic devices.

polymerisation of epichlorohydrin, glycidol and similar bifunctionally reactive monomers giving polyhydroxy polymers.

Polyvinylpyrrolidone (PVP), polyacrylamides, polymethacrylamides etc are examples of polymers in which there are a plurality of amide groups.

Further suitable hydrophilic polymers are reaction products (adducts) between ethylene oxide, optionally in combination with higher alkylene oxides or bisepoxides, or tetrahydrofuran, and a dihydroxy or polyhydroxy compound as illustrated with glycerol, pentaerythritol and any of the polyhydroxy polymers referred to in the preceding paragraphs.

The non-ionic hydrophilic polymer may have the same structure as described for the extenders defined in Berg et al (WO 9833572) ~~which is hereby incorporated by reference~~. In contrast to Berg et al there is no imperative need for the presence of an affinity ligand on the hydrophilic polymer used in the present invention.

One or more positions in the non-ionic hydrophilic polymer may be utilized for attachment. In order to make the hydrophilic polymer flexible the number of attachment points should be as low as possible, for instance one, two or three positions per polymer molecule. For straight chain polymers, such as lower alkylene oxide polymers similar to polyethylene oxide, the number of attachment points is typically one or two, with preference for one.

Depending on the position of a coated part surface within a microchannel structure, the hydrophilic polymer may carry an immobilized reactant (often called ligand when affinity reactions are concerned). Depending on the particular use of a microchannel structure such reactants can be so called